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Regulation of Atherosclerosis and Associated Risk Factors by Adenosine and Adenosine Receptors

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Abstract

Adenosine is an endogenous metabolite that has an anti-inflammatory effect across the vasculature. Extracellular adenosine activates four G-protein coupled receptors (A1, A3, A2A, and A2B) whose expression varies in different cells and tissues, including the vasculature and blood cells. Higher levels of adenosine are generated during stress, inflammation, and upon tissue damage. Some of the adenosine receptors (AR), such as the A2BAR, are further upregulated following such stresses. This review will discuss the role of adenosine and adenosine receptors in the development of atherosclerosis and some of the risk factors associated with this pathology. These include adenosine receptor-regulated changes in atherosclerosis, blood pressure, thrombosis, and myocardial infarction.

Keywords

adenosine; adenosine receptors; foam cells; atherosclerosis; myocardial infarction; ischemic preconditioning

Introduction

The beginning of adenosine research dates back to 1929 when scientists from the University of Cambridge injected intravenously tissue extract from heart, which led to an immediate decrease in heart rate, impaired heart conduction, and arrested experimentally induced arrhythmia of the auricle [1]. The scientists were Drury and Szent-Gyorgi, and the metabolic substance causing these effects on the vasculature and blood flow was adenosine. Adenosine can either be transported out of the cell or generated as a result of catabolic break down of adenine nucleotides during stress [2•, 3], inflammation [4], and tissue damage [5, 3, 6-9]. Released adenosine affects the behavior of the surrounding cells by activating four G-protein coupled receptors. Two of the adenosine receptors (AR), A2A and A2B, couple with $G_{\alpha s}$, activate adenylate cyclase, and increase intracellular 3′-5′-cyclic adenosine monophosphate (cAMP) levels. Whereas, A1 and A3, couple with $G_{\alpha i}$, inhibit adenylate cyclase, and decrease cAMP levels. All of the AR's are activated by nanomolar concentration of adenosine, with the exception of the A2BAR, which in many cases requires

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micromolar concentrations [10], or possible oligomerization with the A2AAR [11]. Expression of these receptors varies in different cells throughout the vasculature and in the blood. Atherosclerosis is a disease of the large and medium arteries and its ultimate outcome is viewed as the number one killer in United States. Atherosclerosis is a result of a complex network of processes typically involving a high lipid profile, and hemodynamic factors in the blood circulation that interact with the innate immune system in the arterial wall [12-14]. Diets rich in cholesterol and saturated fats, lack of exercise, smoking, and high blood pressure all contribute to the development of atherosclerosis and to its impact on the arterial wall.

This review describes the role of adenosine and its receptors in atherosclerosis, and in the development of other cardiovascular events that contribute to atherosclerosis or are amplified by it.

Foam cells and adenosine receptors

Diets rich in cholesterol and saturated fatty acid intake combined with genetic factors lead to an increase in circulating cholesterol and triglycerides, and a consequent activation of the vascular endothelial layer [15]. Endothelial cell activation is characterized by increased expression of cell surface adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule (ICAM1), P-selectin, and E-selectin. Upregulation of adhesion molecules results in recruitment and attachment of mononuclear leukocytes, some of which are monocytes and neutrophils [16]. The attachment and locally produced chemoattractant molecules, such as monocyte chemotactic protein (MCP-1) [17], stimulate monocyte migration into the sub-endothelial space, where they differentiate to macrophages [13]. The differentiation process, which is governed by macrophage colony stimulating factor 1 (MSF-1), subsequently results in upregulation of macrophage scavenger receptors and internalization of lipoprotein particles. The result is accumulation of large droplets of lipids and cholesterol esters, leading to the foamy appearance of macrophage cells and the formation of foam cells [13]. Large accumulation of foam cells in the intima leads to fatty streaks along the artery. Fatty streaks that continue to be exposed to high levels of lipids and inflammation give rise to atherosclerosis.

As foam cell enlargement and accumulation progresses, hypoxia and inflammation increases and adenosine levels rise [18]. Adenosine receptors are expressed on the surface of the endothelial cells, on vascular smooth muscle cells [5] as well as on the surface of different type of leukocytes including macrophages [19]. Released adenosine, however, can render its anti-inflammatory effect on the vasculature by activating the A2 adenosine receptors (A2A, A2B). Endothelial cells lacking the A2BAR have elevated levels of ICAM-1, P-selectin, and E-selectin [5]. Signals coming from either bone marrow-A2BAR or A2AAR lower the levels of vascular adhesion molecules and reduce inflammation [20, 5]. There is no clearly defined role for adenylate cyclase-inhibitory adenosine receptors in the regulation of adhesion molecule expression in vascular endothelial cells, although renal corticies, post renal ischemic injury, show an increase in the message for ICAM-1 and TNF-alpha in the A1AR knockout (KO) mice [21]. Thus, it seems that adenosine receptors affect the initiation of plaque formation by reducing inflammation and adhesion molecule expression. These observations suggest a protective role of adenosine signaling in the endothelial layer primarily through the A2-type adenosine receptors, and possible delay in early atherosclerotic events.

The monocyte/macrophage lineage represents a crucial player in the development of atherosclerosis. All four of the adenosine receptors are expressed on the macrophage surface [18], and macrophages are major contributors to overall systemic inflammation. Activation

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of the A2AAR in this lineage leads to elevation of the anti-inflammatory cytokine IL-10 and to reduced levels of pro-inflammatory cytokines such as TNF-alpha [22]. In accordance, macrophages depleted of the A2BAR secrete much higher levels of TNF-alpha and IL-6 [5]. In vitro studies using murine [23] and human macrophage [24] cell lines (J774.1 and U937 respectively) demonstrate that A3AR activation inhibits LPS-stimulated TNF-alpha production. This result is confirmed in vivo [25], suggesting that the adenylyl cyclase-inhibitory receptors can also have anti-inflammatory function.

As a result of its anti-inflammatory properties, adenosine provides protection against foam cell formation and development of atherosclerosis. In activated human THP-1 monocytes cell line and in murine peritoneal macrophages activation of the A2AAR by specific ligand inhibits foam cell formation, as antagonism or lack of this receptor has no effect [26, 27]. Mechanistically activation of the A2AAR reduces foam cell formation by increasing reverse cholesterol efflux through elevated levels of the lipoprotein transporter ABCA1 [28•]. Reiss et al., observed an effects on foam cell formation in peritoneal macrophages expressing or lacking the A3AR [25], as the KO cells could not reduce foam cell formation upon stimulation or inhibition with A3AR specific ligands [26]. Interestingly, in the human macrophage cell line U937, pharmacological inhibition of the A3AR (with MRE 3008F20) and antagonism of the A2BAR (with MRE 2029F20) inhibited foam cell formation [18], suggesting further attention to the interpretation of cell lines studies in this field of research.

Advanced vascular lesions and adenosine receptors

Advanced vascular lesions begin with accumulation of vast amount of foam cells that start to necrotize and initiate T-cell infiltration to the intima. T-cells, together with neutrophils, contribute to the signaling mechanism that drives the vascular smooth muscle cells (VSMC) to move into the intima. There, VSMC de-differentiate, proliferate and start secreting extracellular matrix proteins. The result of this process is a formation of a fibrous cap around the necrotic foam cell mass (reviewed in [13]). No one has yet determined whether adenosine levels in or around the vascular lesion are elevated. On the other hand, inflammation rises as atherosclerosis progresses, and inflammatory cytokines increase expression of adenosine receptors [19, 29, 4]. Indeed, studies done in mouse models lacking adenosine receptors show that the role of adenosine in atherosclerosis, whether protective or deleterious, depends on the ablated receptor.

The first glimpse of the role of adenosine in atherosclerosis started with the elimination of the A3AR on an Apolipoprotein E (ApoE) null background. ApoE KO mice are a useful model to study atherosclerosis, since mice do not naturally develop all stages of atherosclerotic lesions along the arterial tree [30-33]. In the case of the A3AR, although aortic vascular smooth muscle cells exhibit decreased proliferation potential upon receptor-elimination and A3AR contributes to the process of inflammation, there is no effect on the chronic development of atherosclerosis [34].

As mentioned earlier, A2AAR KO macrophages have increased foam cell formation potential and A2AAR is anti-inflammatory. It is surprising, however, that A2AAR, ApoE double KO mice on high fat diet have smaller atherosclerotic lesions, as compared to the control mice used in the study. The major contributors to this anti-atherosclerotic profile were the macrophages; in vivo these cells were highly apoptotic and vascular lesions were smaller [35•]. Overall, elimination of the A2AAR results in increased levels of plasma cholesterol, concentrated predominantly in the LDL particles [35•]. These findings are quite intriguing, considering that increased inflammatory state and elevated plasma cholesterol typically lead to atherosclerosis. Yet the above study shows that the A2AAR is deleterious, rather than protective against atherosclerosis. Further, in a model of restenosis post arterial

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angioplasty, elimination of the A2AAR results in an increase of neointima and raised the possibility that A2AAR is actually protective against vascular lesion formation [36•]. In this case, in the A2AAR KO, there was an increase in the homing ability of leukocytes to the sub-endothelial space, which led to a consequent increase in neointima thickness [36•]. It is quite possible that under chronic inflammation (atherosclerosis) A2AAR is harmful, but under more acute conditions (post angioplasty) it becomes protective. This defines the A2AAR signaling more complex than expected, and this receptor might be more challenging as therapeutic target in the context of vascular disease.

The role of the A2BAR in vascular diseases has also been extensively studied. As described earlier, endothelial A2BARs regulate the expression of surface adhesion molecules and improve leukocyte rolling [3]. In a similar fashion to the A2AAR, in a model of restenosis post angioplasty, the A2BAR protects against increased neointima formation. This protective effect is due to A2BAR-mediated signals coming from bone marrow cells [3]. Vascular smooth muscle cells lacking expression of this receptor have an increased proliferation rate [3], and activated macrophages show an increase in levels of matrix metalloproteinase-9 (MMP-9) levels [37]. Vascular-derived matrix metalloproteinases, such as MMP-9 play an important role in neointima formation after vascular injury and in de-stabilizing advanced atherosclerotic plaques [37].

All of these in vitro and in vivo studies are consistent with a protective effect of the A2BAR against vascular diseases. In a model of atherosclerosis, where the A2BAR is eliminated in ApoE null mice, plaque formation along the aortic tree is elevated. Risk factors, such as plasma cholesterol and triglycerides, detrimental in the development of atherosclerosis, are highly augmented when this receptor is eliminated. This lipid elevation of cholesterol and triglycerides is predominantly concentrated in the very low density lipoprotein (VLDL) particles. Consistently, activation of the A2BAR with specific agonist (BAY 60-6053, Bayer, Germany) leads to reduction in lesion formation, and a decrease in synthesis and plasma levels of cholesterol and triglycerides. It is important to mention that the activation of the A2BAR is beneficial with respect to atherosclerosis and plasma lipids even during high-fat, high-cholesterol diet [2•] (Fig.1). The major organ contributing to the anti-atherosclerotic profile, in the case of this receptor, is the liver. Normally the liver expresses very little A2BAR [3], but with high fat, high cholesterol diet the levels of A2BAR in this organ become vastly elevated [2•]. Mechanistically, activation of the A2BAR in hepatocytes in vivo and in vitro causes a decreased activation of the transcription factor sterol regulatory element binding protein one (SREBP1), a major switch regulating lipid synthesis.

In summary, with respect to atherosclerosis, A2BAR is protective against atherosclerosis and its activation reduces vascular lesion formation. In contrast, A2AAR elimination is protective and A3AR has no effect (Fig.1). The role of A1AR in this regard is not known.

Adenosine and thrombosis

The rupture of an advanced atherosclerotic lesion can lead to an impairment of vessel blood flow, giving rise to myocardial infarction [38]. As a result, there is exposure of matrix proteins, such as collagen that can result in platelet accumulation and cross-linking, creating a thrombus that can give rise to acute coronary events. Platelet activation can contribute to atherosclerosis and other vascular disease, while excessive thrombosis can increase the risk of a pathological outcome in this vascular disease. In the pathogenesis of a myocardial infarction, platelets are a major player and adenosine and adenosine receptors are known to affect platelet function. Ex vivo studies using platelets isolated from A2AAR KO mice have shown that these cells exhibit mildly higher aggregation [39] and platelet-platelet interaction, which is important for the formation of tertiary structure of the clot. The

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mechanism involved is cAMP-dependent. Consequently, ligand-specific activation of the A2AAR on human platelets results in an increase in cAMP levels and consequent decrease in platelet aggregation [40]. More recent studies have shown that the other cAMP-elevating receptor, the A2BAR, has a modest effect on aggregation as well. In this case, platelets isolated from the A2BAR KO mice have decreased levels of cAMP levels and elevated expression of the ADP receptor P2Y1, which is a potent activator of aggregation [41]. Platelets do not express the A1AR or A3AR [42]. Thus, in case of thrombosis, adenosine protects against excessive thrombus formation through the A2-type adenosine receptors.

Adenosine and myocardial infarction

Myocardial infarction occurs when the blood flow in the heart is impaired due obstruction caused by rupture of atherosclerotic plaque and or thrombi development around that plaque. This obstruction often leads to ischemia and consequently to impaired oxygen supply, which, if untreated, can lead to cell death and damage to the heart. Restoration of blood flow and oxygen supply or reperfusion post myocardial infarction causes significant injury to the tissue and elevated inflammatory response [43]. The inflammatory signals in the course of reperfusion originate from cardiomyocytes and endothelial cells, and these cells express A1AR and A2-type ARs, respectively. Ischemia leads to decreased oxygen supply that causes hypoxia and affects endothelial function by augmented cellular permeability and induction of procoagulant properties [44]. There is also a decrease in cAMP levels as a result of reduction of endothelial adenylate cyclase activity [45]. Adenosine, in turn, regulates levels of cAMP in vascular endothelial cells through its receptors.

Studies examining the role of selective A2AAR agonist (ATL-146e) on myocardial infarction, using a canine model, show that infarct size is significantly reduced upon activation of this receptor. In the infracted myocardium of ATL-146-treated dogs there is reduced accumulation of neutrophils [46]. Neutrophils are the major contributor to cardiac damage by in this process by causing damage or apoptosis of the tissue and impaired endothelial function [47]. This suggests that adenosine has a beneficial effect on reduction of myocardial infarction, and this effect is mediated by the A2AAR. Adenosine signaling has also a beneficial effect on ischemic preconditioning, a process that renders myocardium resistant to the detrimental effects of ischemia [43]. In this setting, activation of the A1AR results in reduction of infarct size, as inhibition of A1AR with specific antagonist (8-cyclopentyl-1,3-dyproxylxanthine) blocks the protective effect of preconditioning. The mechanism by which A1AR exhibits its protective effect during preconditioning is by opening the myocardial K_{ATP} channels [48]. Selective activation of A3AR by Cl-IB-MECA (2-chloro-N(6)-(3-iodobenzyl)adenosine-5′-N-methylcarboxamide) also protects against myocardial ischemia/reperfusion injury [49]. Recently, Eltzschig's group has shown that, at least in mice, A2BAR is the major receptor that acts as protective in ischemic setting [50]. During ischemic preconditioning, the A2BAR is selectively upregulated, and from all of the AR KOs, A2BAR KO mice are the only ones in which this process is not protective [50]. Injection of A2BAR specific agonist (BAY 60-6583) significantly reduces infarct size after ischemia [50]. These studies suggest that adenosine, signaling mostly through the cAMP-elevating A2AAR or A2BAR plays an important protective role during ischemic preconditioning and can decrease myocardial infarction.

Adenosine and blood pressure homeostasis

Atherosclerotic plaque becomes destabilized as a function of weakened fibrous cap. This destabilization is a complex balance among lipid content in the plaque, distribution of vascular smooth muscle cells, shear stress coming from the blood flow, and weakened endothelium [51, 52]. High blood pressure as a result of narrowing of the arteries due to the

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plaque formation has been associated with high risk of vulnerable plaque rupture and consequent predictor of myocardial events.

Adenosine, in turn, is beneficial against blood pressure elevation, and it can decrease heart rate (reviewed in [53]). Studies in rats have shown that adenosine exhibits its effect on heart rate and homeostasis of blood pressure (baroreflex) by acting on the brain Nucleus Tractus Solitarii (NTS) [54]. In this part of the brain, activation of extracellular signal-regulated kinases 1,2 and the endothelial nitric oxide synthase (eNOS) are the downstream targets of adenosine, possibly through the A2AAR [54]. Additionally, adenosine signaling through the A2AAR [55], and the A2BAR [56], provides a potent vasodilatory effect on mean arterial pressure. In the vessel, endothelial A2AAR leads to an increase in nitric oxide (NO) production as a result of activation of the eNOS [57].

More recently, it has been shown that adenosine activation of the A2AAR can lower mean arterial pressure and cause tachycardia independently of eNOS [58]. In cardiomyocytes, adenosine increases levels of NO in eNOS-dependent manner, and prevents mitochondrial damage [59]. Mice that lack the A2AAR exhibits an increase in blood pressure [39] and increase [39] or decrease of heart rate [60], depending on the background of the strain from which the gene was knocked out (Table I). An interesting observation related to A2AAR has been reported during hemodialysis in humans. The process causes an increase in adenosine with concomitant increase in expression of A2AAR in peripheral blood mononuclear cells and a consequent adverse hypotension [61]. Thus, targeting the A2AAR could be a valuable tool for lowering blood pressure. The other major adenosine receptor involved in blood pressure homeostasis is the A1AR [56, 62]. Activation of cardiac A1AR reduces cAMP levels, hyperpolarizes the K_{ATP} channels and increases potassium levels. The result of this signaling cascade leads to transient block of the Atrioventricular node (AV) and a consequent reduction in blood pressure [63]. A1AR null mice have elevated blood pressure at baseline on low sodium diets [64]. Elimination of the A1AR also results in an increase in heart rate [60]. These studies have been done using the A1AR KO model (created by Fredholm's group [65]) and using implant transmitter in the carotid artery on either mixed background (C57BL,129/OlaHsd) [64], or in the same mice outbred to C57BL/6J [66]. Blood pressure measured by tail cuff method in an A1AR KO created by Schnermann group (mice on C57Bl/6J) showed no significant difference between A1AR KOs as compared to wild type controls [62]. It is possible that tail cuff method is not sensitive enough to account for transient changes, or the background of the mouse strain and disguises the effect that adenosine might have on blood pressure.

The role of the A3AR or the A2BAR on blood pressure homeostasis, if any, is not comparable to the effect of the A1AR and the A2AAR. Elimination of either of these former receptors has no effect on blood pressure measured by tail cuff method in twelve week old mice [34, 2, 5]. Although there is no effect on blood pressure, A2BAR KO mice (on C57BL/6J background) have significantly reduced heart rate as compared to wild type after seven weeks on high fat diet [2•]. This influence of adenosine on heart rate regulated by the A2BAR could be a consequence of the action of this receptor in the brain, which is yet to be explored. Further, it is possible that signaling of adenosine through the A2BAR has a modest effect on blood pressure, which is not detected by the less sensitive tail cuff method..

In summary, adenosine acting simultaneously in the central nervous system, and in the periphery can maintain favorable blood pressure levels and thus provide a beneficial effect against plaque rupture.

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Conclusion: Adenosine receptors as therapeutic targets for cardiovascular disease

There is no doubt that adenosine has a crucial role in the development of atherosclerosis, myocardial infarction and blood pressure homeostasis. All four receptors can potentially be beneficial targets for different aspects during the pathogenesis of cardiovascular disease. Agonists of A1AR and A3AR could be targeted for regulation of ischemia and preconditioning. However, the first drug designed to specifically inhibit A1AR, Rolofylline (KW-3902, Merk and Co, New Jersey, NJ) for the treatment of compensated heart failure, did not give a favorable outcome. There was increased neurological outcomes in the treated group versus placebo, as well as increased incidence of stroke [67]. Adenosine receptor polymorphism was partially responsible for the undesired response to this drug, as infarct size was associated with three variants in the 3′-untranslated region of A1 and one in the coding region of A3AR [68].

Based on mouse studies, one might envision development of antagonists for the A2AAR to halt the development of atherosclerosis. On the other hand, A2AAR being a potent vasodilator, could be targeted for lowering blood pressure using an agonist (rather than antagonist). Indeed, an A2AAR specific agonist has been developed to cause vasodilatation. The agonist, also known as Regadenson (Astellas Pharma Inc, Deerfield, IL) causes an increase in blood flow to different tissues enabling myocardial perfusion imaging [69]. A2AAR agonists can be used to inhibit platelet activation and thrombosis.

If mouse studies translate to patients, targeting of the A2BAR could turn to be most beneficial, because of its uniform protective effect on atherosclerosis and thrombosis. Activation of this receptor by specific agonist reduces atherosclerosis without change of western diet intake. That is, the pathology can be reversed rather than just being prevent it without modifying life style. Additionally, cholesterol and triglyceride levels decrease upon receptor activation, as synthesis of liver lipids drops down. The A2BAR activation is a potent reducer of ischemia during myocardial infarction. Thus, future development of A2BAR agonists would be initial steps towards examining the therapeutic potential of this receptor in humans with respect to atherosclerosis and cardiovascular disease.

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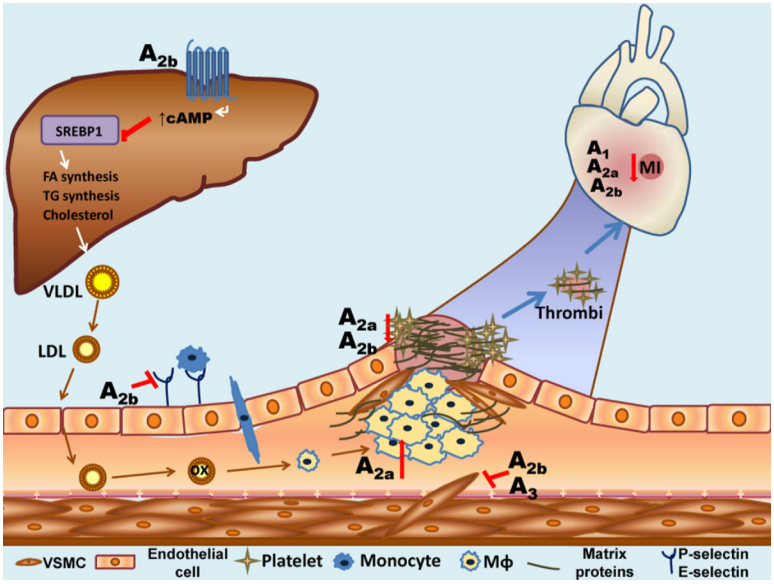

Figure 1. Adenosine receptors and their beneficial role in atherosclerosis and cardiovascular disease

Western diet (high fat, high cholesterol) increases expression of A2BAR in the liver. Activation of this receptor by a specific agonist reduces processing of SREBP-1, leading to decreased synthesis and plasma levels of cholesterol and triglycerides. In the vasculature, A2BAR activation has a beneficial effect on reducing the expression of adhesion molecules (E-selectin, P-selectin, ICAM-1) responsible for leukocyte/monocyte recruitment. In the endothelial subspace, where lesions begin to form, A2AAR inhibition in macrophage/foam cell signals to induce early apoptosis, and as such reduces vascular lesion development. Both A2AAR and A2BAR provide second layer of protection in the intima and in the circulation by inhibiting inflammatory cytokine release. A2BAR activation also reduces matrix metalloproteinase levels, and as such could potentially stabilize the plaque. Upon plaque brake off, A2AAR and A2BAR reduce platelet aggregation and thrombus formation. Finally, in the heart, A2AAR activation reduces myocardial infarction, and A1AR, A3AR, and A2BAR act protective in the process of ischemia reperfusion. Abbreviations: VSMC, vascular smooth muscle cell; Mφ, macrophage/foam cell; Ch, cholesterol; TG, triglycerides; FA, fatty acids; VLDL, very low density lipoprotein particle; LDL, low density lipoprotein particle; ox on LDL, oxidized LDL

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Table I

Adenosine receptors KOs and their contribution to atherosclerosis and related vascular outcomes

Mouse model	Background strain	Lipid profile/ foam cell potential	Blood pressure/ HR	Atherosclerosis	Effect on Platelet function
A1AR KO	C57BL,129/OlaHsd [65]	ND	↑BP [64]	ND	ND
	C57BL/6J [65, 62]	ND	↑BP [66], No effect on BP [62]	ND	ND
A1AR, ApoE dKO	---	---	---	---	---
A3AR KO	C57BL/6/B6D2 [25]	↓foam cell formation [26]	No effect (tail cuff) [42]	No effect [34]	No effect [42]
A3AR, ApoE dKO	C57BL/6 [25, 34]	ND	ND	No effect [34]	ND
A2AAR KO	CD1 [39]	ND	↑BP; ↑HR [39]	ND	↑Aggregation [39]
	C57BL/6NTac [27]	↑Cholesterol/LDL [35] ↑Foam cell formation [26]	↓HR [60]	No effect [35]	ND
A2AAR, ApoE dKO	C57BL/6NTac [27],[35]	↑Cholesterol/LDL [35]	ND	↓Aortic plaque formation [35]	ND
A2BAR KO	C57BL/6J [5]	↑Cholesterol [2] ↑TG[2]	No effect on BP [5] ↓HR [2]	No effect [2]	↑Aggregation [41] (modest effect)
A2BAR, ApoE dKO	C57Bl/6J [2]; [5]	↑Cholesterol/VLDL [2] ↑TG/VLDL [2]	No effect on BP or HR [2]	↑Aortic plaque formation [2]	ND

The table specifies the mouse strains used, as this property contributes to the interpretation of the results obtained, as exemplified here with blood pressure studies in A1AR KO mice on different backgrounds. For instance, C57BL/6 sub-strains of mice originating from different companies have certain genetic modifications that result in phenotypic differences. Mice from Taconic (C57Bl/6 also known as C57Bl/6NTac) as compared to Jackson's lab mice (C57BL/6J) have at least 12 microsatellite differences [70], and an important deleterious mutation in the nicotinamide nucleotide transhydrogenase (Nnt) gene [71]. Nnt is an enzyme responsible for the mitochondrial interconversion of NADH to NADPH. This mutation and lack of the enzyme in Taconic C57BL/6 mice has been associated with impaired normal mitochondrial function, such as protection of oxidative stress, in addition to increased insulin secretion, impaired glucose homeostasis [72], behavioral impact [73], fertilization [74], and susceptibility to infection [75]. In that sense, it is quite possible that the background of the strain is important in determining differences in blood pressure, and it calls for using the appropriate strains as controls. **ND**, not determined; **HR**, heart rate; **KO**-knockout; **dKO**, double KO; **---**, no publications available;